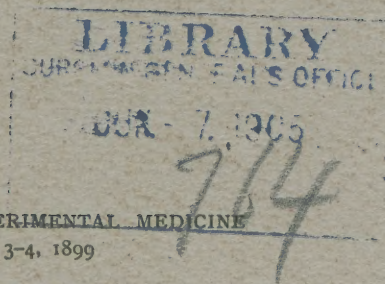


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(FROM THE PATHOLOGICAL LABORATORY OF THE RUSH MEDICAL COLLEGE)



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PLATES XII-XV.

Since the description by Gilchrist,† in 1894, of blastomycetic dermatitis, and the report by Gilchrist and Stokes‡ of the second case, two more instances have been recorded, one by my assistant, H. G. Wells,§ which like the two previous cases resembled lupus vulgaris clinically, and the other and fourth by R. Hessler.|| The latter's case began as a pimple, which gradually enlarged and formed a small abscess, from the contents of which a blastomyces was cultivated. After evacuation the abscess healed, but at the time of the report new foci were making their appearance in the neighborhood of the old scar. The only other of the four instances of blastomycosis of the skin, in which the organism has been isolated in pure culture, is that of Gilchrist and Stokes, who record full observations on the cultural, morphological, and

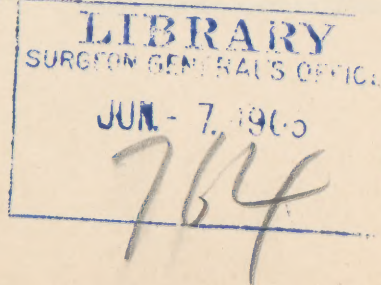
* Read by title at the meeting of the Association of American Physicians, Washington, D. C., May 1-3, 1899.

† *Johns Hopkins Hospital Reports*, 1896, i, 269.

‡ *Johns Hopkins Hospital Bulletin*, 1896, vii, 129; *Journal of Experimental Medicine*, 1898, iii, 53.

§ *New York Medical Journal*, March 26, 1898.

|| *Indiana Medical Journal*, August, 1898.



pathogenic properties of the *Blastomyces dermatitidis*, which is the name they gave the fungus in their case.

It is now proposed to describe briefly the results of the histological and biological examination in the case of blastomycetic dermatitis recently under the care of Professors Hyde and Bevan, in the Presbyterian Hospital, of Chicago, so as to add to the number of instances illustrative of the action of pathogenic blastomycetes—microorganisms which of late are attracting considerable attention.

I am under many obligations to Professor Hyde, who saw the case first and referred it to Professor Bevan, for the privilege of making the following abstract of the clinical history:*

R. B., by occupation a day laborer, 56 years of age and married, presented himself for treatment September 20, 1898. He was a native of Holland; at 18 years of age he left for the East Indies, where he entered the Dutch army, serving in the East. He was then taken ill with some form of fever, which resulted in his leaving the East Indies for his native land. He remained in Holland for eight or nine years after, and came to America in 1892.

His present malady began four years ago by the development of a small reddish spot in the right leg, the disorder gradually extending over its anterior part. For this trouble he underwent some operation in the Dearborn Hospital; he was also treated at the Hahnemann Hospital. The result was the production, in the region named, of a thin, broad, cicatriform tissue, insensitive, and in places scaling.

One year ago the left thumb and wrist were found to be involved in some morbid process, which produced by gradual extension from the point of original implication a reddish patch, well defined in outline, with a bluish-red areola and a decidedly verrucous aspect. This patch furnished a moist and sticky exudate. The subjective symptoms were a sense of soreness and burning, at times decided pain and pricking sensations. The pain often radiated up the arm, so that both the elbow and shoulder of the affected side became sensitive. The fingers became partially flexed toward the palm; the little finger was thus permanently flexed.

*The clinical aspects of this case are fully dwelt upon in an article by Hyde, Hektoen and Bevan (*A Contribution to the Study of Blastomycetic Dermatitis, British Journal of Dermatology*, 1899, xi). Here is also a brief report of a new case of blastomycosis of the skin of the leg which I discovered in a museum specimen.

He suffered from headaches, which were frequent and severe; from constipation, and from inappetence. His sleep was poor. His height was 5 feet 9 inches; weight, 98 pounds, a decline from his average weight of 145 pounds. He used tobacco in moderation but no alcoholic stimulants, though he had once been a heavy drinker.

His father died at the age of 92, in good health; his mother at 45, of consumption. There were three brothers living, one dead from consumption; three sisters were dead of consumption, at 21, 16, and 48 years of age, respectively. He had had six children; four were living and in good health, two were dead, one at 39 months, the other at 2½ years, from causes unknown.

When examined by Prof. Hyde the region of involvement of the right leg extended in an irregular oval, its long axis parallel with the axis of the extremity, from the lower margin of the upper third of the anterior surface of the limb quite to the lower border of the lower third, its lateral wings barely perceptible when the posterior surface of the leg was viewed from behind. The encroachment upon the calf was about equal on the two sides, the outer wing slightly more elevated than its fellow. This entire surface was covered with a thin, papery substitute for the normal epidermis, which furnished a slight scale.

The dorsal aspect of the proximal phalanx of the right thumb and of the wrist, with a portion of the posterior face of the lower third of the forearm, were involved in a single connected patch of morbid tissue, dull-reddish in hue, sharply defined in outline and having a peculiar bluish-red border extending about 4 mm. away from the edge of the affected area. The body of the patch was made up of an elevated mass of infiltration, with a verruciform development of fine, pin-head-sized papillæ projecting externally and moistened by a glutinous secretion. Individual elements of this warty growth were readily distinguished by the eye. The palm was completely spared. There was entire absence of other lesions, such as pustules, vesicles, crusts, or the scar-like tissue exhibited on the leg. No other signs of disease were discovered.

For the purpose of further examination and study of the lesion presented by the patient, he was placed in the Presbyterian Hospital, under the charge of Professor Bevan who, on September 27, 1898, excised a small piece of the upper margin. Frozen sections, stained with hæmatoxylin and eosin, showed extensive epithelial proliferations and cell infiltrations in the corium; in the new-formed epithelial masses were numerous miliary abscesses, in one of which I found two round bodies, composed of an outer bluish membrane, an inner colorless and hyaline layer,

and faintly stained granular contents with a few blue bodies like cocci. A diagnosis of blastomycetic dermatitis was accordingly made.

Soon afterward Professor Bevan placed the patient on iodide of potassium, with the result that material diminution in the size of the patch occurred, and such marked amelioration of all the symptoms that the question of operative interference was for the time set aside. The patient had been taking the drug named before he first came under Professor Hyde's observation, but probably not in such doses as those administered at the hospital.

Microscopical Examination.—Portions of the bit of tissue excised September 27 were fixed in absolute alcohol. On October 6 Professor Bevan, at my request, excised a long, narrow strip from the centre of the lesion on the back of the hand, the clinical appearance of which now indicated rapid healing, especially at the margins. Pieces from this strip were fixed in absolute alcohol, sublimate and Zenker's fluid. Paraffin sections were stained with hæmatoxylin and eosin, Gram's method preceded by carmine, and alkaline methylene-blue, with or without counterstain with eosin.

The histology of the skin lesion may be summarized as follows: It can be said at the outset that the histological appearances in this case are identical with those described by Gilchrist and by Wells. In fact their descriptions would answer very well for this case also.

The surface of the skin is covered by a horny layer of varying thickness, together with debris, polymorphonuclear leucocytes, blood discs and bacteria, especially bacilli that take Gram's stain. In places small abscesses have broken through the horny layer, which lies directly on the granular or prickle layer. The striking feature in the sections is the great hyperplasia of the epithelium, very similar to rapid carcinomatous proliferation, and in the form of variously shaped and branching downgrowths of from 3 to 5 mm. in extent, which start from the inter-papillary processes principally and cause great distortion of the papillary layer. In the larger columns of epithelial cells the central parts may show degeneration as well as distinct hornification. Isolated epithelial whorls, more or less completely hornified, also occur. The epithelial proliferations are generally separated from the fibrous tissue of the corium by a quite distinct layer of cylindrical epithelial cells, next to which occur typical prickle cells. Occasionally karyokinetic figures occur in the epithelial cells.

Scattered throughout the entire epithelial portion are polymorphonuclear leucocytes, which occur between as well as within the cells. Nu-

merous focal collections of leucocytes, which when larger form miliary abscesses, are found in all parts of the thickened epithelium, in the deep prolongations, near the surface as well as directly underneath it, and at times they have broken through externally. The contents of these characteristic abscesses consist of leucocytes, with more or less nuclear fragmentation, desquamated epithelial cells, detritus of horny material, red blood corpuscles, and the organism peculiar to the process; in some are also multinuclear giant cells of the tuberculous type. The epithelial cells around the abscesses seem to play an entirely passive role and to be flattened out by the pressure of the abscess contents.

The connective tissue between the epithelial downgrowths presents typical acute and subacute inflammatory changes: the vessels are congested, leucocytes are seen in the act of migration, there are hæmorrhagic extravasations as well as oedematous districts. The cell infiltration is marked in many places, but there are but very few miliary abscesses and no organisms have been found in the corium; a few giant cells are present; endothelioid cells, plasma cells, and polymorphonuclear leucocytes make up the principal part of the infiltration; the mast cells are rather numerous. In places young connective tissue has formed. Occasional typical hyaline bodies, mostly as mulberry-shaped masses, are present. In one instance a large cell with a nucleus like that of plasma cells was filled with small hyaline spheres.

As to the appendages of the skin present it would seem that they play but a passive role; the coils of the sweat glands are crowded apart by the inflammatory infiltration, and the hair follicles are the seat of a marked hyperkeratosis.

The organisms in this case do not seem to be present in the tissues as numerous as in the cases previously reported. This impression is gained after carefully looking over a large number of sections from various parts of the area involved. All the mature organisms seen occur in the miliary abscesses in the epithelial proliferations (Figs. 1 and 2, Plate XII), most often singly, sometimes in groups of from two to four; they are invariably situated outside of the cells; not even the giant cells in this case contain any parasites. The parasites, when not budding, are round or oval, and about 10 to 12 mikrons in diameter; they are surrounded by a homogeneous capsule, from which the finely granular protoplasm is separated by a clear zone of varying width; in some the protoplasm contains a vacuole of varying size, which in a

few instances occupies the larger part of the cell, crowding the granules closely together inside the clear zone, which becomes indistinct; in a few organisms the outer capsule contains oblong thickenings. Budding bodies in varying stages are present; the granular protoplasm pushes the capsule and clear zone before it, forming an oval bud which grows larger and eventually separates from the mother organism. Methylene blue gives the best stain of the parasite, the capsule assuming a deep blue color, the protoplasm a lighter blue. There are no red granules in the parasites when stained in this way, as described by Gilchrist.

*Cultures.**—Smears of soft material from the bit of skin first excised contained a few bacilli and a very small number of round bodies similar to those found in the sections. Three glycerine-agar and three blood-serum tubes were inoculated. The resulting colonies consisted of a bacillus, mixed with a few large round bodies; attempts at isolation of the latter by means of the plate method failed. Two attempts at cultivating yeasts from the hand yielded pure growths of a pathogenic bacillus which belongs in the pseudo-diphtheria group. Numerous cultures were also made from the strip of skin removed October 6; small pieces of tissue were rubbed over the surface of potato, glycerine-agar and glucose-agar; inoculations were also made in Pasteur's fluid. After 24 hours there was a heavy growth upon all the solid media, consisting of a comparatively few large clear bodies and bacilli. Isolated by the plate method the bacilli proved to be pseudo-diphtheria bacilli already found, and an unidentified bacillus; the plates also showed some colonies which consisted of round bodies of variable size; in the subcultures these bodies were so closely and constantly associated with small coccus-like organisms that for a time grave doubts were felt as to the purity of the growth. In subcultures on glycerine- and glucose-agar there were noticed after several days colonies with two distinct shades of color, namely a creamy yellow and an almost pure white. Now, from the yellow part the smears showed only small, round or oval, often apparently budding and deeply stained organisms, often not larger than large cocci, while the white colonies consisted of much larger, mostly quite clear round bodies, varying in diameter from 7 to 10 mikrons.

Inoculations of the smaller organism in the yellow colony on fluid media were followed in two or three days by the growth of a fine film on

*I am indebted to Mr. H. E. Davies for the successful isolation of the blastomyces in pure culture.

the surface, which consisted of small bodies, while the slight deposit at the bottom contained mostly larger forms. Inoculations on bouillon with the larger form gave the same results. Cultures of the smaller organisms on glucose- and glycerine-agar at room temperature developed both forms, smears showing long chains of more or less deeply stained small bodies which were closely connected, and at one end of the chain there would often be one or more of the large clear organisms. It was also soon noticed that when a culture of the larger form became dry the organism seemed gradually to become smaller. It was therefore concluded that it concerned one and the same organism, which in the course of artificial growth appeared in various sizes.

This organism grows readily on all of the following media, the extent and rapidity of growth corresponding roughly to the order in which they are named, beginning with the most favorable: beerwort-agar, glucose- and glycerine-agar, Löffler's blood-serum, potato (alkaline), glucose- and glycerine-broth, lactose-, dextrose-, and saccharose-broth, agar-agar, gelatine, milk, and Pasteur's fluid.

Agar and Gelatin Plates.—On glycerine-agar plates pin-point-sized and even larger colonies develop in 24 hours at 35° C.; they are roundish and oval, rather coarsely but evenly granular and very light grayish in color. With a moderate magnification the individual yeast bodies are readily distinguishable, especially at the margins, where budding forms often occur (Fig. 3, Plate XIII). As the colonies become larger the central part turns more opaque; the surface colonies are larger than the deep.

The deep colonies on gelatin plates show the individual, clear, circular bodies very distinctly, while the surface growths are smaller, irregular in outline, more opaque, and composed of much smaller organisms. In the hanging drop colonies of gelatin small, opaque buds are seen between the large, clear organisms. Generally the process of budding results in the growth of small round colonies, but sometimes peculiar, long outgrowths of two or more quite parallel rows of bodies develop, so that the colony becomes more or less club-shaped.

Hanging-drop cultures of bouillon show the multiplication by budding very well, and also the structure of the organism (Fig. 4, Plate XIII).

Agar.—The organism grows most luxuriantly upon 5 per cent beerwort-agar. On slanting wort- and glycerine-agar there develops in 24 hours an extensive, grayish or yellowish-white growth, without any special characteristics and which spreads itself over the surface and, especially in the case of the beer-wort-agar, gradually creeps in between the

medium and the glass. With time the growth becomes perhaps more purely white; it remains quite soft and smooth on the surface. In from 8 to 14 days quite abundant, fluffy and feathery masses have grown down into the agar from the under surface of the superficial growth; this is more marked on the beer-wort-agar. These peculiar feathery or downy outgrowths are roundish or cone-shaped, the large ones being as much as 3 mm. in diameter; they are very much like the downgrowths characteristic of many varieties of ray fungi.

In stab cultures the surface is soon covered by a spreading, slightly raised smooth layer. The growth along the needle track becomes quite thick and presents irregular, nodular, sometimes almost polypoid protuberances, which give it a peculiar twisted appearance. In a few days this knobby trunk presents delicate lateral branches springing from various parts of the circumference and passing out into the medium at right angles to the stem, after the fashion of the branches of certain trees. These feathery, often extremely fine, lateral outgrowths resemble very much the tree-like branchings of stab cultures of many forms of actinomyces.

On agar-agar the organism produces a rather light yellowish-brown pigment, which smears show to be in the form of small granules that occur about and upon some of the larger clear bodies. This property of pigment production is not observed upon any other media and develops, to a moderate degree, on transplantation upon plain agar from whatever other medium it may be, but it is not absolutely constant.

Gelatin.—The growth on gelatin is rather scanty. There develop a flat, thin, pearly surface layer and an irregularly granular tapering growth along the needle track, with peculiar lateral outgrowths consisting of delicate tufts, straight or branching threads of varying length and thickness; some of the threads break up into an extremely fine terminal network at some distance from the main stem. Gelatin is not liquefied.

Serum.—On Löffler's serum the growth is quite extensive.

Potato.—On alkaline potato there forms in 24 hours a slight yellowish-white, raised growth of considerable extent; with time this increases materially, the margins being rounded and irregularly wavy. On acid potato the rate of growth is much slower.

Bouillon.—In bouillon there forms a granular, rather coarse, sometimes a little stringy sediment and gradually small granular masses appear scattered throughout the fluid, which at first is quite clear. Sometimes a clear film develops on the surface.

Milk.—Moderate growth occurs in milk, without any change in reaction and without caseation.

Fermentation Tests.—The organism grows fairly well in the aerobic bulb of fermentation-tubes containing lactose-, dextrose-, and saccharose-bouillon, made from sugar-free bouillon, prepared according to Theobald Smith's formula,* but without fermentation. It also grows to a limited extent in Pasteur's fluid. It does not produce indol.

Reaction to Oxygen.—The organism does not grow in Buchner's jars.

Temperature.—The thermal death-point is 54° C., an exposure to this temperature for two minutes killing the organism. It is not killed by freezing; cultures exposed to a temperature just above zero for two weeks and then frozen solid for two weeks, on inoculation gave rise to typical new colonies.

Relation to Potassium Iodide and the Pseudo-diphtheria Bacillus.—The rapid improvement of the local lesion under the use of iodide of potassium suggested the advisability of determining the effect if any of the presence of this drug in the media inoculated. It was found that a 1 per cent iodide of potassium bouillon furnishes just as favorable a medium for the growth of the organism as ordinary broth.

It also occurred to us that possibly the mixed infection with the pseudo-diphtheria bacillus referred to might play some role in the rapid healing of the lesions. The blastomyces was found to grow unhindered in the presence of the bacillus in the same media; it develops just as rapidly in filtered two weeks' old bouillon cultures of the pseudo-diphtheria bacillus as in the ordinary broths.

Morphology.—The size, shape, and structure of the organism as it develops in artificial culture vary somewhat in the different media and with age. Some reference to this variability has already been made in connection with the account of the early efforts to obtain pure cultures. Fresh specimens mounted in salt solution, from the culture of glycerine-agar, show a highly refractive organism with a doubly contoured membrane.

The organism is not destroyed by caustic potash. It stains readily with the common aniline solutions, but the stain is rather deep and in many cases too diffuse and the clearest pictures are obtained by a rather prolonged staining—15 to 30 min.—of carefully made films in a .5 per cent solution of methylene-blue and then washing well with water. The films are made by suspending a small quantity of the culture in a drop of physiological salt solution or of distilled water and drying. This stain brings out the different parts of the cells whereas other methods stain diffusely.

* *Journal of Experimental Medicine*, 1897, ii, 546.

Large bodies, such as seen in the tissues of the hand as well as in those of infected animals, are not constant in the cultures. However, as the organism begins to grow on most of the common solid media and under favorable circumstances, it generally presents an outer well-defined but thin membrane, which is separated from the protoplasm proper by a clear and transparent zone. The size varies between 7 and 12 μ . The form is round or oval, sometimes polygonal on account of mutual pressure. Budding forms are very frequent and occur in all stages. The process of budding seems to begin with the appearance of a small projection of the endosporium, which pushes the transparent zone and outer membrane in front of it; very soon these layers enclose the new bud fully and the point of attachment to the mother cell may be either flattened or, later, drawn out into a slender pedicle. When the buds are small it is not always possible to recognize the various parts mentioned. In the fairly well developed bodies the endosporium is vacuolated and, generally, finely granular; not rarely some of the granules may be coarse, of varying size, and deeply stained, sometimes producing appearances that resemble the presence of a nucleus, but this is not constant. The vacuole, which may be large or small, is as a rule centrally located. Sometimes there is no vacuole; this is generally the case in young, small buds and newly separated bodies. As the culture becomes older the number of really large bodies with large vacuoles seems gradually to increase; the protoplasmic granules, which also become larger, are then either crowded to one side or arranged as a rim around the vacuole; in many of these bodies the transparent zone is no longer distinctly recognizable. Such large bodies are surrounded by numerous much smaller ones, sometimes vacuolated, sometimes not. In some preparations these bodies seem to lie in a gelatinous material. In a glycerine-agar culture five weeks old or more large bodies are usually quite numerous. In some cultures the size of the cells, the extent of vacuolation, etc., are quite uniform, in others rather variable. The uniform size, regular and characteristic form and large vacuoles of the cells in cultures obtained from an extensive and richly cellular exudate produced by inserting a quantity of yeast-cells into the anterior chamber of a rabbit's eye have persisted unchanged through many generations grown on glycerine-agar.

Large bodies with huge vacuoles are also prone to form in cultures on Löffler's blood serum (Fig. 5, Plate XIII, and Figs. 6 and 7, Plate XIV).

With Gram's stain some organisms remain deeply and diffusely stained while others are partly decolorized, frequently showing, however, three or more deeply colored granules at or upon the margins of the bodies.

By means of stained microscopic sections of the stab cultures in glucose-agar and gelatine it is readily determined that the peculiar lateral outgrowths and branchings observed in these cultures do not depend upon the formation of mycelium but are composed of budding round forms only. This peculiar way of growing is therefore identical with the development of the curious club-shaped colonies in the gelatine hanging drop.

In liquid media, especially bouillon and Pasteur's fluid, as well as, but to a much less extent, on potato and other solid substance, the organism may be comparatively small. It appears that the buds become separated while still minute. Not rarely a parent cell is found surrounded by a number of minute free as well as attached buds, many of which are so small as to resemble cocci. Now, these small bodies, which, as a rule, stain diffusely and homogeneously, may give rise to succeeding generations of small organisms, varying in size from 1 to 5 μ . Repeated budding without complete separation may give rise to chains and groups of various lengths and sizes. Certain parts of smear preparations from cultures in Pasteur's fluid and bouillon may resemble very much those of a medium-sized micrococcus, except that the yeast fungus may present rather pronounced variations in size (Fig. 8, Plate XIV). When the minute forms cluster closely around large, vacuolated bodies the first impression that it concerns a mixed culture is certainly not surprising.

In some cultures a distinct mycelium develops. This is most noticeable in Pasteur's fluid and bouillon, but mycelioid growth-forms may occur to a very slight degree in all media, *e. g.*, plain agar. The formation of mycelium is due apparently to a gradual elongation of individual organisms of the smaller type, resulting in the early stages in irregularly cylindrical-shaped bodies which later grow out into either curved or fairly straight, rather thick rods of varying lengths or, more rarely, form long coiled threads. Buds may arise from any part of the mycelium and they may be sessile or pedunculated. When buds separate from the ends of a rod and grow in length or when all the members in a chain of organisms begin to elongate, a segmented mycelium is formed; the appearance between the ends of two segments of new buds which also grow out long, or the dislocation of a segment from its position in the mycelium, may give rise to quite typical false ramifications. The cylindrical masses, the rods and threads vary in thickness, the average being about 5 mikrons. For the most part the substance of the mycelium is solid and dense, staining diffusely, but parts occur in which there is a membrane enclosing an empty space containing in places finer or coarser granules (Fig. 9, Plate XV).

Cultures on agar-agar are frequently characterized by the production of a granular, yellowish-brown, at times also reddish, pigment. Here are found medium-sized typical bodies, quite a few oblong and elongated narrow, diffusely stained forms but no typical mycelium, and also a considerable number of round bodies covered and surrounded by yellow or yellowish-brown pigment granules which are quite uniform in size (Fig. 10, Plate XV). There is no pigment about the long forms. In the early stages of pigment formation the granules appear in the immediate vicinity of the outer capsule, both within as well as outside it. As the amount of pigment about an organism increases, the endosporium disappears—a fact pointing to a pigmentary degeneration of these bodies. The rather limited growth on plain agar has already been mentioned.

ANIMAL EXPERIMENTS.—1. A minute fragment of the first bit of skin was inserted into the peritoneal cavity of a guinea-pig, which died a month later, greatly emaciated. There were no changes at the point of inoculation or in the peritoneal cavity, cultures from the small amount of clear fluid in which remained sterile. The organs contained in pure form the pseudo-diphtheria bacillus, previously isolated from the hand. The liver was extensively necrotic and cirrhotic, but these changes are attributable to the bacillus solely.

2. A white rat was inoculated subcutaneously with 1.5 cc. of a bouillon culture. A small local abscess formed. The animal died in ten days. The abscess cavity contained a few cc. of a whitish-yellow viscid pus, from which the blastomyces grew in pure culture. The internal organs were sterile.

Microscopic examination of the abscess wall shows a thin capsule of young fibrous tissue covered by a quite thick layer of pus cells and nuclear detritus, among which rather small, doubly contoured, circular bodies resembling blastomycetes are seen. The liver contains a few minute foci of typical granulation tissue composed of plasma cells, endothelioid cells, small giant cells and polymorphonuclear leucocytes, but it is not possible to find any typical organisms in these areas. There are quite a number of small, similar foci in the lungs, especially around the bronchi, and in the giant cells of these areas occasional rather typical, round bodies are observed. The other organs are normal.

3. A gray mouse died five days after receiving 1 cc. of a bouillon culture subcutaneously. The abscess which had formed contained the organism in pure culture, while the internal organs were normal.

4. A medium-sized rabbit died 48 hours after subcutaneous inoculation of 2.5 cc. of a bouillon culture. Cultures from the internal organs

remained sterile. There was an extensive coccidiosis of the liver. There were numerous minute foci in the lungs composed of epithelioid and giant cells, as well as leucocytes with considerable nuclear degeneration. In some of the giant cells were circular bodies resembling the organism injected, as well as small, round bodies that stained rather diffusely with methylene-blue, presenting a faint peripheral transparent zone. (There is no record of the condition at the site of the inoculation.)

5. A white rat received 1.5 cc. of a bouillon culture. Viscid, yellow pus formed about the point of inoculation and the organism was reclaimed in pure culture. The animal died five days after the injection. The internal organs contained the *Staphylococcus albus*.

6. A gray mouse was inoculated subcutaneously with 1 cc. of a bouillon culture. It died in five days. The small abscess that had formed contained the organisms, as well as *Staphylococcus albus*; the latter organism was present in all the internal organs.

7. A small part of a colony on agar was inserted into the anterior chamber of the left eye of a large male rabbit. There gradually developed a hypopyon, with softening and threatened destruction of the cornea. The animal was killed after two weeks. The internal organs, microscopically healthy. The anterior chamber filled to distention with a semisolid, yellowish-gray material, which when spread out on cover slips and treated with hydrate of potassium contained numerous spherical bodies. Inoculations with this material on glycerine-agar gives a rich and pure growth of the blastomyces. Cultures from other organs sterile. Sections of the eye show the anterior chamber to be filled with cellular detritus, among which are a number of round, deeply stained bodies surrounded by a rather faint halo, but no distinct outer membrane. The lens is largely softened and the iris is the seat of a diffuse cellular infiltration. The iris also contains rather small, deeply stained, round bodies which at times are surrounded by minute, hyaline-like formations that take the methylene-blue stain very intensely.

8. A small bit of a yeast culture on glycerine-agar was inserted into the anterior chamber of the eye of a large white rabbit. The anterior chamber rapidly filled with a white exudate, the cornea became opaque, and a mucopurulent conjunctivitis developed which soon ceased. Three weeks after the inoculation the cornea had become quite opaque and vascularized, and the anterior chamber contained a large, roundish, yellow mass which has since slowly enlarged. At present, February 27, 1899, the eye is of about normal size, apparently blind, and the yellow growth does not show any signs of diminution. The rabbit seems to be in good general health.

9. A guinea-pig received 1 cc. of a bouillon culture in the abdomen. It remained well, and was killed five weeks later. There were numerous small, grayish-yellow, translucent areas in the lungs; the other organs were healthy. Cultures from all the internal organs remained sterile. Microscopically the areas in the lungs consisted of foci of granulation tissue without any giant cells, but with extensive nuclear fragmentation. Only a few round bodies were to be found, after prolonged search in numerous sections, stained with methylene-blue, as well as by other means.

10. A white rat received 1 cc. of a bouillon culture in the abdomen. It died thirty-four days later. The lungs contained pin-head sized and larger areas of a grayish, rather soft appearance. Smears from these, treated with caustic potash, showed numerous large, round, doubly contoured bodies. The microscopic sections showed extensive bronchopneumonic areas of an inflammatory tissue with marked nuclear fragmentation and quite a number of large round bodies, which stained diffusely. (Unfortunately no cultures were made in this case.)

11. A medium-sized rabbit was injected with 0.5 cc. of a bouillon suspension through the ear vein. Considerable swelling resulted, which healed after three weeks with marked deformity. The animal, which seemed well, was killed 37 days later. The lungs contained a few small nodules; the liver was the seat of a marked coccidiosis. Cultures from all the organs remained sterile. Sections from the lungs show miliary foci of young granulation tissue, but typical blastomycetes are not to be found.

12. A medium-sized dog was injected with 2 cc. of a bouillon suspension into the peritoneal cavity. The animal remained well and was killed 40 days later. All the organs were normal and sterile.

13. A large black rabbit received into the circulation 4 cc. of a bouillon suspension of a culture on beer-wort-agar. It died during the following night. The lungs were oedematous, the thymus ecchymotic, the liver swollen, soft and mottled; the spleen and the kidneys appeared normal.

Bacteriological Examination.—Smears from the various organs show numerous clear, round bodies not destroyed by KOH; they are most plentiful in the smears from the lungs and kidneys.

Inoculation from the various organs on glycerine-agar give rise to numerous colonies of blastomyces, most numerous in the tubes from the kidneys. The cultures from the liver and spleen also contain the colon bacillus. Only one colony of budding fungi developed in the tube from the heart's blood.

Histological Examination.—The lungs are extremely congested and the capillaries contain many polymorphonuclear leucocytes; in the alveolar walls are numerous small foci of cell accumulation with marked nuclear fragmentation and quite typical blastomycetes are found in the interior of some of these areas of necrosis; the alveoli and bronchi contain a finely granular material in which lie occasional round homogeneous bodies.

The liver and spleen show no special changes.

The kidneys show quite extensive changes in the glomeruli, consisting in a granular disintegration of smaller or larger portions of some of the capillary tufts and of a more or less pronounced general accumulation of granular detritus in the subcapsular space, the cells in the lining of which are generally well preserved. The epithelium of the convoluted tubules is very granular and the lumen is filled with granular material. The vessels are generally congested. Lying in the glomeruli, the intertubular vessels, and in the granular material are a few oval or round bodies about as large as a red blood corpuscle, which stain diffusely with the gentian-violet of Gram's method.

14. February 15, 1899, a guinea-pig, weighing 392 grammes, received in the abdomen 12 cc. of a bouillon culture of the blastomycetes heated to 59° C. for 1 hour. It became rapidly emaciated; on February 17 it weighed 340 grammes, on the 20th 285 grammes, on the 22nd 250 grammes; it died on the 22nd. There was marked emaciation but no microscopic lesions in any of the organs or tissues; cultures from the organs and from the heart's blood remained sterile.

The organism above described differs considerably from the *Blastomycetes dermatitidis* of Gilchrist and Stokes, which so far is the only blastomycetes of similar origin with which to make comparisons. Hessler's brief description in his preliminary report on the organism isolated by him from a small cutaneous or subcutaneous abscess only contained a few general statements. Our organism grows much more rapidly than the one of Gilchrist and Stokes, the formation of mycelium is not nearly so marked as in their cultures, in which were not seen the peculiar down- and out-growths and lateral branchings nor the pigment formation (on agar-agar) characteristic of the blastomycetes now described. Both organisms correspond, however, in their action on gelatin, which is not liquefied, in the non-production of indol, and in the complete absence of fermentation of various sugars. Morpho-

logically they are also quite or nearly alike. Gilchrist and Stokes make no mention of such great variability in the size as observed in our organism.

With regard to the pathogenic action of these two organisms attention may be called to the great similarity, amounting to perfect identity, in the histological changes produced by them in the human skin. The marked epithelial hyperplasia, the diffuse more or less chronic inflammatory processes associated with the formation of giant cells and of the characteristic miliary abscesses in the epithelium and elsewhere in the skin, together with the presence, especially in the abscesses, of the round, double contoured, budding organism, constitute the histological picture of blastomycetic dermatitis as now understood. Gilchrist and Stokes found that the blastomyces isolated by them would produce nodules of a chronic inflammatory nature when inoculated into the dog, horse, sheep, and guinea-pig, whereas white mice and rabbits seemed immune. Our organism may be said to be pathogenic to rabbits, guinea-pigs, white rats, and gray mice. It is probably harmless to the dog, as far as can be judged from our intraperitoneal inoculation of this animal.* Its local action may be characterized as necrotic and leucotactic, associated with or followed by the growth of an inflammatory granulation tissue, corresponding in the latter respect to the majority of the pathogenic yeasts studied by various Italian investigators, by Lydia Rabinowitsch, and others. Its general action may be regarded as slowly toxic, leading after a varying length of time to death from marasmus, and, as experiment No. 14 would seem to show, the dead cultures possess an apparent marantic effect. It corresponds therefore very closely to those varieties of pathogenic blastomycetes which, according to Casagrandi,* pro-

* Since writing the above, the following experiment has been made: On March 22, 8 cc. of a bouillon suspension of blastomycetes were injected into the jugular vein of a small dog, which died greatly emaciated April 17, the autopsy and microscopic examination showing minute foci of granulation tissue throughout the lungs and softened, cellular areas with yellowish contents in the medullary pyramids of the kidneys. The blastomycetes were recovered in pure growth and in large numbers from the lungs and the kidneys. No growth of any kind resulted from the inoculation on glycerine-agar and blood serum of 1 cc. of the heart's blood.

* Ueber die pathogene Wirkung der Blastomyceten, *Centralbl. f. Bakt., Abth. I*, 1898, xxiv, 754.

duce local necrotic or suppurating foci or permanent nodules and a fatal marasmus. It can probably not be said that the toxic and marantic effects of these forms of blastomycetes have been so pronounced as to have been definitely recognized in the few carefully observed instances of human blastomycetic infection so far recorded, but certainly the marked decline in weight noted by Prof. Hyde in this case is very suggestive of this effect and is therefore in full accord with the experimental observations.

That certain blastomycetes may produce more distinctly suppurative changes in the skin and also elsewhere in man, as well as in animals, has been clearly shown in the now classical case of Busse,* by Hessler's case, and in the experimental study of the question of suppuration from yeasts by Nesczadimenko.† In connection with this, reference may also be made to the instance of refractory subcutaneous abscesses caused by a fungus possibly related to the *Sporotricha* described by Schenck.‡

To return to the consideration of the blastomycetes studied by Gilchrist and Stokes and by me, it might be regarded as quite clearly demonstrated from some of the foregoing considerations that the clinical and histological pictures of blastomycetic dermatitis, which from these aspects would appear as a distinct entity, may be produced by organisms which differ so much in certain cultural and pathogenic characteristics that they must be regarded as separate, though closely related, varieties. In view of the present unsatisfactory status of classification of the blastomycetes it is desirable to refrain from becoming dogmatic. Casagrandi, for instance, found it quite impossible to classify the blastomycetes upon either morphological or biological grounds on account of the pronounced variability of the individual forms.

* The dermatological aspects of Busse's case have been fully considered by Busche in an article entitled "Ueber Hautblastomykose" (*Verhandl. d. VI. Deutschen Dermatolog.-Congresses*). Unfortunately this article came to my notice too late to be considered at this time.

† *Zur Pathogenese der Blastomyceten*, *Centralbl. f. Bakt.*, Abth. I. 1899, xxv, 55.

‡ *Bulletin of the Johns Hopkins Hospital*, 1898, ix, 286.

DESCRIPTION OF PLATES XII-XV.

(Photographs by Dr. W. H. Knap.)

PLATE XII.

Fig. 1. A miliary abscess in the epithelium of the hand, containing in its upper half a group of three organisms. $\times 220$.

Fig. 2. The three organisms in Fig. 1, more highly magnified. $\times 1500$.

PLATE XIII.

Fig. 3. A colony of the organisms on glycerine-agar. $\times 250$.

Fig. 4. The organism as seen in a hanging-drop culture of bouillon. $\times 1000$.

Fig. 5. Vacuolated and solid diffusely stained organisms from glycerine-agar culture. $\times 1000$.

PLATE XIV.

Fig. 6. Budding organisms. Gentian-violet. $\times 1000$.

Fig. 7. Large vacuolated and small solid bodies from a blood-serum culture 5 weeks old. $\times 1000$.

Fig. 8. Chains of the minute form. $\times 1000$.

PLATE XV.

Fig. 9. The development of mycelium with sessile and pediculated buds. $\times 1000$.

Fig. 10. The development of pigment granules around and upon some of the larger cells in cultures on plain agar. Several elongated forms are present. $\times 1000$.

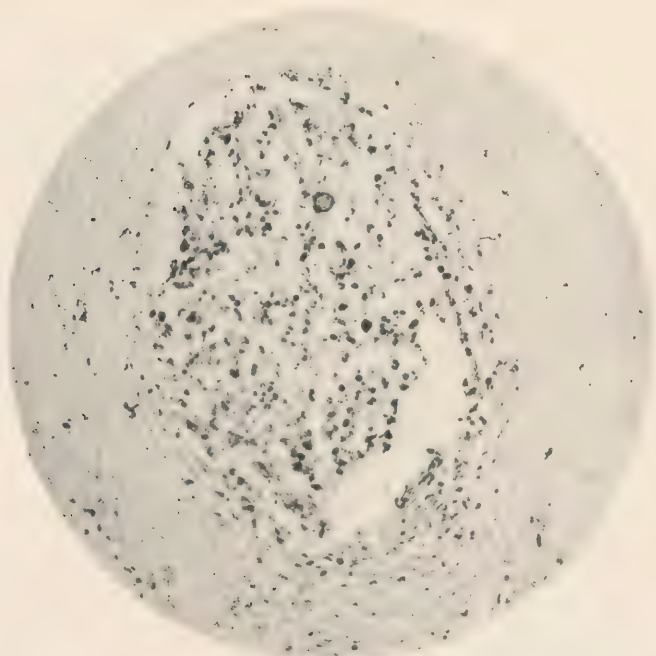


FIG. 1.

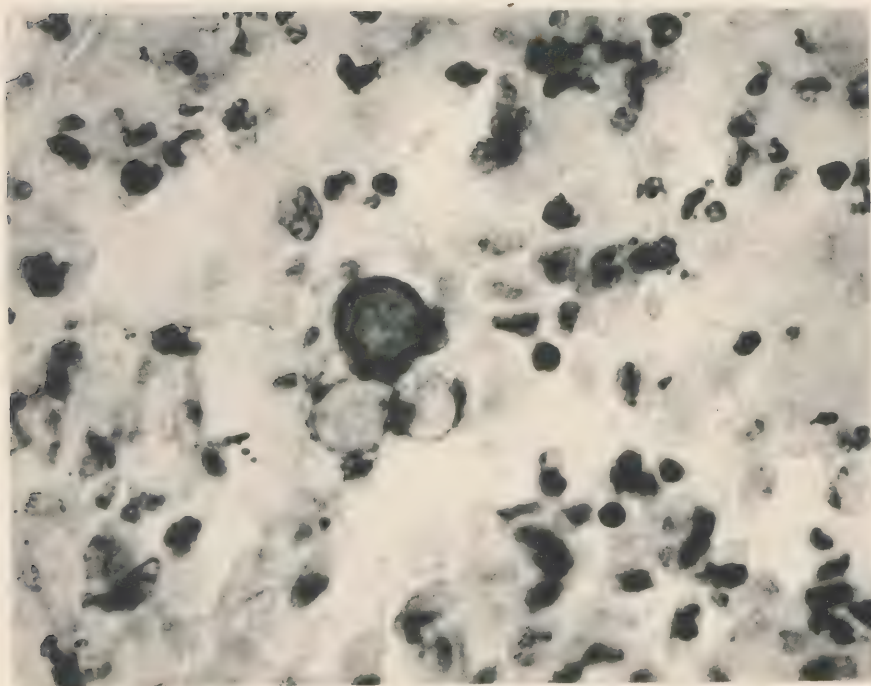


FIG. 2.

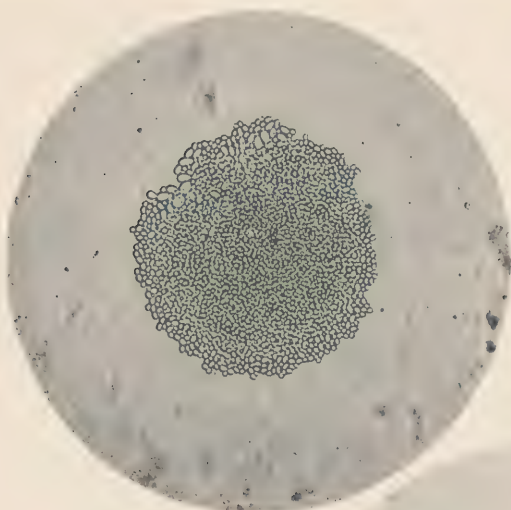


FIG. 3.

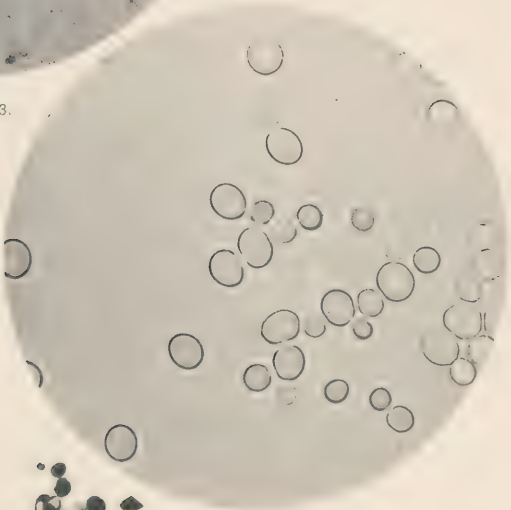


FIG. 4.

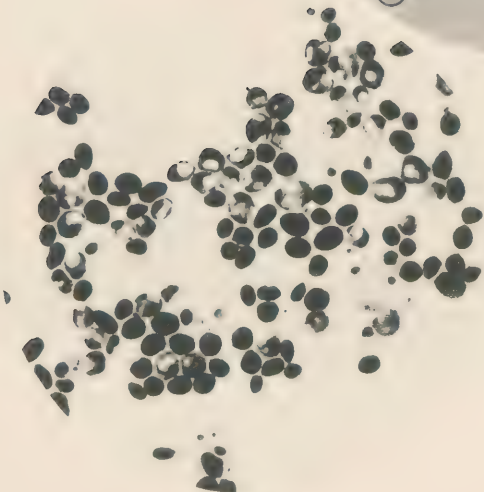


FIG. 5.

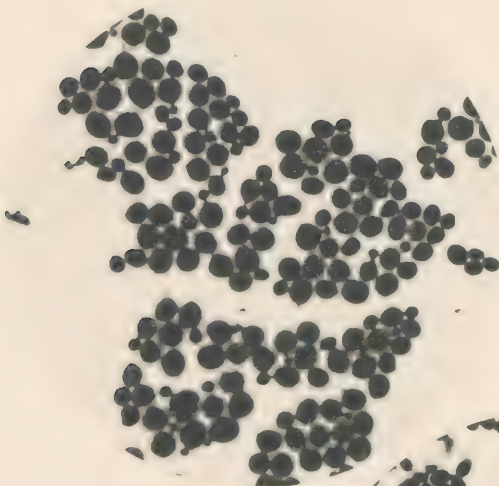


FIG. 6.

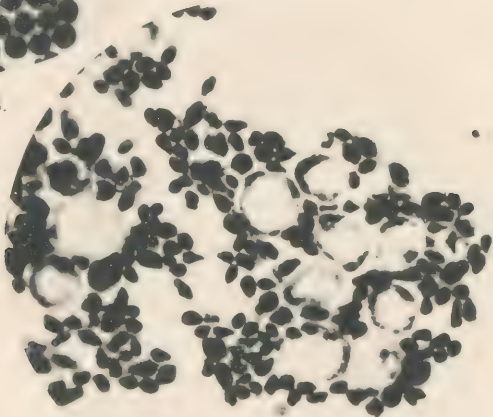


FIG. 7.

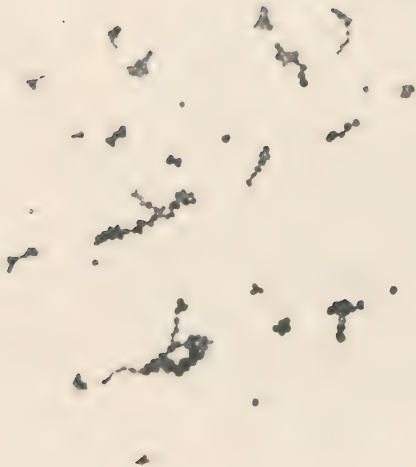


FIG. 8.

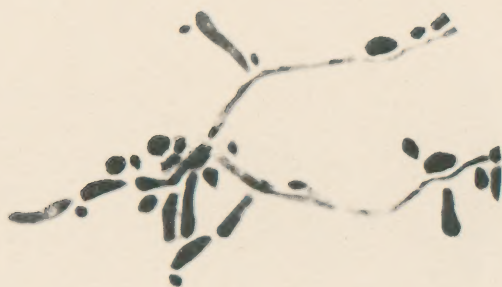


FIG. 9.

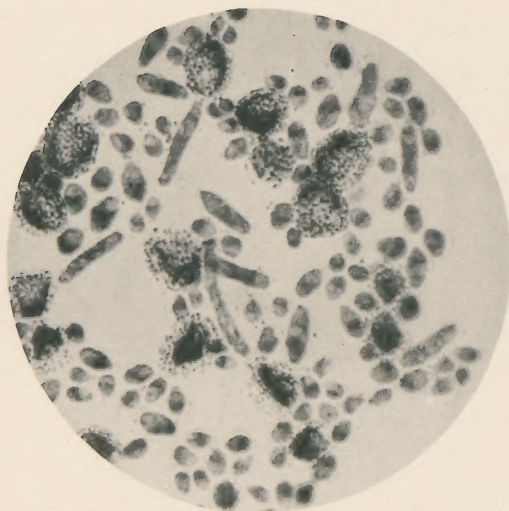


FIG. 10.

